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(19) (CA) APPLICATION FOR CANADIAN PATENT (12)

(54) Optical Sensor for the Determination of Cations

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Notice: This application is as filed and may therefore contain an incomplete specification.



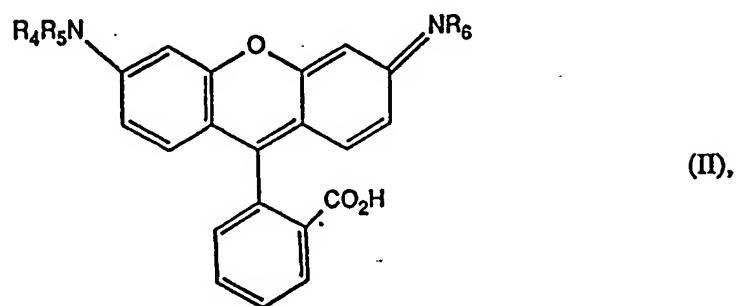
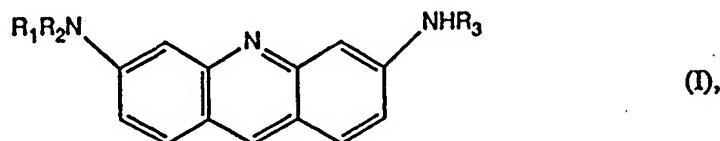
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Optical sensor for the determination of cationsAbstract

Compounds of the formulae I and II



in which R₁ and R₃, and R₄ and R₆ are C₁-C₃₀alkyl or C₁-C₃₀alkyl-CO-, and R₂ and R₅ are H or C₁-C₃₀alkyl, with the proviso that the total number of carbon atoms in the alkyl groups is at least 12, and salts thereof with inorganic or organic acids are fluorophores of high basicity which can be used in membranes for the optical determination of cations in aqueous media, in particular potassium ions, through the measurement, in particular, of the concentration-dependent reduction in fluorescence.

Optical sensor for the determination of cations

The invention relates to a sensor for the optical determination of cations from the group consisting of metal and ammonium cations (referred to as cations below) in aqueous samples by the fluorescence method, which sensor contains certain highly basic dyes from the group consisting of rhodamines and acridines as fluorophores in the active coating, and to a process for the qualitative or quantitative determination of cations, in particular in aqueous solutions, using the optical sensor. The invention furthermore relates to certain acridines and rhodamines and to the use thereof as fluorophores in sensors for the optical determination of cations in aqueous samples.

The optical determination of metal cations has recently achieved increased importance, the presence or concentration of metal cations being measured, for example, via the change in absorption or fluorescence of a suitable dye. The sensors known as optrodes generally comprise transparent support material and an active coating. This active coating generally contains a transparent hydrophobic polymer and a lipophilic plasticizer for achieving adequate ion diffusion and solubility of the active constituents. The active constituents are a specific ionophore as complexing agent for metal cations, a lipophilic salt as counterion for maintaining electrical neutrality, and an indicator substance which, due to a chemical change or a physical change in the environment, generates a measurable optical signal.

US-A-4 645 744 describes systems of this type in which the indicator substance is a neutral compound, for example a dye (p-nitrophenol), which interacts with an ionophore/metal cation complex, causing a colour change as the optically measurable signal. The interaction can cause, for example, the elimination of a proton from the dye, causing a change in the electron state. Suitable compounds include fluorescing compounds (for example fluorescein), whose fluorescence changes due to the change in the electron state and can be determined optically by means of fluorescence measurements.

H. He et al. in Chemical, Biochemical and Environmental Fiber Sensors II, SPIE Vol. 1368, pages 165 to 174 (1990), describe systems containing a proton carrier (Nile Blue) as

indicator substance, in which the transport of potassium into the active coating by means of valinomycin as ionophore causes dissociation of the proton carrier and diffusion of a proton into the aqueous phase. The proton carrier changes its colour from blue to red and, depending on the choice of wavelength, either the reduction in fluorescence of the blue dye or the corresponding increase in the fluorescence of the red dye can be determined. Due to the higher sensitivity and selectivity, measurement of the fluorescence is preferred. A significant disadvantage of the process is the low sensitivity of the system, due to the low fluorescence quantum yield of the indicator dye used.

J.N. Roe in Analyst, Vol. 115, pages 353 to 358 (1990), describes a system based on energy transfer due to complex formation of the fluorescence dye used with the anionic form of a certain indonaphthol, which itself forms a ternary complex with the potassium-charged ionophore. The potassium is determined by measuring the change in absorption after charging with potassium or from the change in fluorescence. The sensitivity and response speeds of this system are regarded as unsatisfactory.

Y. Kawabata in Anal. Chem. Vol. 62, pages 1528-1531 and 2054 to 2055, describes a membrane system for the optical determination of potassium using a hydrophobic ion exchanger, namely 3,6-bis(dimethylamino)-10-dodecyl- or -10-hexadecylacridinium bromide. A change in fluorescence is achieved by changing the polarity in the microenvironment of the sample, since the acridinium salts diffuse at the interface with the aqueous phase due to ion exchange with the potassium ion.

W.E. Morf et al. in Pure & Appl. Chem., Vol. 61, No. 9, pages 1613 to 1618 (1989), describe the use of pH-sensitive chromo- or fluoroionophores for the optical determination of cations based on ion exchange reactions. The sensitivity of these systems is relatively low, the measurement is hindered in optically dense systems, and relatively high concentrations of chromo- or fluoroionophores in the membrane are required.

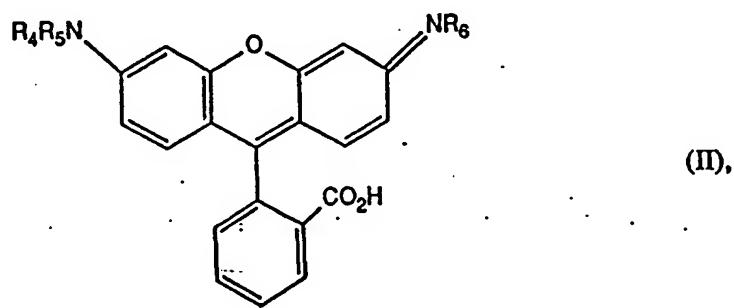
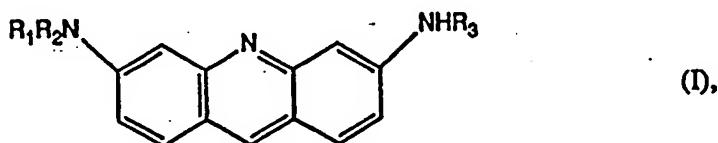
K. Wang et al. in Analytical Science, Vol. 6, pages 715 to 720 (1990), describe membranes containing an absorption dye (Nile Blue) as indicator substance for the optical measurement of metal cations. The system is based on an ion exchange mechanism which reduces the absorption by protonation of the dye. The sensitivity of the system is regarded as too low.

Hitherto, no systems having an ion exchange mechanism for the optical measurement of

metal cations has been disclosed which are based on the determination of the reduction in fluorescence of a fluorophore and have high sensitivity, since the fluorescence quantum yields and basicities of the known pH-sensitive fluorophores are too low.

It has now been found that certain acridine dyes and rhodamine dyes surprisingly satisfy these high requirements and are lipophilic, pH-sensitive and highly basic fluorophores which are highly suitable, in a neutral polymer membrane together with an ionophore and a lipophilic salt of a borate, for the determination of metal cations, in particular potassium, by the ion exchange mechanism and have a fluorescence which is highly dependent on the corresponding metal cation concentrations. These fluorophores are distinguished by a high fluorescence quantum yield, high basicity, a large difference between the fluorescence signals of the protonated and deprotonated forms, high lipophilicity, adequate photostability and suitable absorption and emission wavelengths. Highly sensitive systems for the optical determination of metal cations on the basis of fluorescence measurements can be provided.

The invention relates to compounds of the formulae I and II



in which R₁ and R₃, and R₄ and R₆ are C₁-C₃₀alkyl or C₁-C₃₀alkyl-CO-, and R₂ and R₅ are H or C₁-C₃₀alkyl, with the proviso that the total number of carbon atoms in the alkyl groups is at least 12, and salts thereof with inorganic or organic acids.

In a preferred embodiment, R₂ is H.

The alkyl groups can be linear or branched and preferably contain 1 to 22 carbon atoms. Linear alkyl groups are preferred. Examples of alkyl are methyl, ethyl and the isomers of propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, eicosyl, heneicosyl, docosyl, tricosyl, tetracosyl and triacontyl. In a preferred embodiment, R₁ and R₃ are C₆-C₂₄alkyl or C₆-C₂₄alkyl-CO-, particularly preferably C₁₀-C₂₄alkyl or C₁₀-C₂₄alkyl-CO-, especially preferably C₁₄-C₂₂alkyl or C₁₄-C₂₂alkyl-CO-, while R₂ is H. In another embodiment, R₅ is preferably H, and R₄ and R₆ are preferably C₆-C₂₄alkyl, particularly preferably C₁₀-C₂₄alkyl, especially preferably C₁₄-C₂₂alkyl. In a further embodiment, R₄ and R₅ are preferably C₁-C₄alkyl, particularly preferably C₁-C₄alkyl, especially preferably methyl or ethyl, and R₆ is C₁₀-C₂₄alkyl or C₁₀-C₂₄alkyl-CO-, preferably C₁₄-C₂₂alkyl or C₁₄-C₂₂alkyl-CO-, especially preferably C₁₆-C₂₂alkyl or C₁₆-C₂₂alkyl-CO-.

The salts of the compounds of the formulae I and II can be derived, for example, from HF, HCl, HBr, HI, H₂SO₃, H₂SO₄, H₃PO₃, H₃PO₄, HNO₂, HNO₃, HClO₄, HBF₄, HPF₆, HSbF₆, CF₃SO₃H, toluenesulfonic acid, C₁-C₄alkyl- or phenylphosphonic acid, formic acid, acetic acid, propionic acid, benzoic acid, mono-, di- or trichloroacetic acid, or mono-, di- or trifluoroacetic acid. Preference is given to HCl, HBr, H₂SO₄, HClO₄, HBF₄, HPF₆ and HSbF₆.

The compounds of the formula I can be prepared in a manner known per se from commercial 3,6-diaminoacridine by stepwise alkylation by means of various alkylating agents or alkylation using one alkylating agent or acylating agent. Examples of suitable alkylating agents are dialkyl sulfates or monohaloalkanes, in particular chloro-, bromo- and iodoalkanes. Examples of suitable acylating agents are carboxylic anhydrides and in particular carboxylic acid halides, for example carboxylic acid chlorides. This reaction can be carried out in the presence of inert polar and aprotic solvents, for example ethers, alkylated acid amides and lactams, sulfones or sulfoxides, and at elevated temperatures, for example from 50 to 150°C. It is expedient to add a halogen scavenger, for example alkali metal carbonates.

The compounds of the formula II can be obtained, for example, by reacting phthalic anhydride with 2 mol equivalents of 2-monoalkylaminophenol. Another possible preparation comprises reacting 2-monoalkylaminophenol with 1 mol equivalent of 2-hydroxy-4-dialkylamino-2'-caboxybenzophenone. These reactions are described, for

example, in US-A-4 622 400. The reaction is expediently carried out in an inert solvent, for example hydrocarbons or ethers. Molar amounts of a condensation agent, for example Lewis acids, concentrated sulfuric acid, perchloric acid or phosphoric acid, are advantageously added. The reaction temperatures can be, for example, from 50 to 250°C.

The compounds of the formula I can be isolated in a conventional manner by precipitation, crystallization or extraction and purified, if necessary, by recrystallization or chromatography. They are crystalline, red, red-brown or red-violet compounds.

The compounds of the formulae I and II are highly suitable as fluorophoric dye indicators for the optical determination of cations in an aqueous environment, in particular by measurement of the change in fluorescence.

The invention furthermore relates to a composition comprising

- (a) a transparent support,
- (b) which is coated on at least one side with a transparent coating which comprises
 - (b1) a hydrophobic polymer,
 - (b2) a plasticizer,
 - (b3) the salt of a lipophilic anion,
 - (b4) an ionophore which forms a complex with the ion to be determined, and
 - (b5) a compound of the formula I or II as fluorophore.

The compounds of the formulae I and II preferably have a pK_a value of at least 8, particularly preferably at least 10.

The support can be formed, for example, from a plastic material, mineral materials or glass and can have any desired shape, for example plates, cylinders, tubes, tapes or fibres. Glasses are preferred.

The thickness of the coating on the support can be, for example, from 0.01 to 100 µm, preferably from 0.1 to 50 µm, more preferably from 0.1 to 30 µm, and particularly preferably from 0.1 to 10 µm.

Various types of polymer are suitable for the composition. They expediently have a mean molecular weight of at least 100 000 daltons, for example from 100 000 to 2 000 000 daltons, preferably from 200 000 to 1 000 000 daltons. The polymers must have

adequate solubility in organic solvents so that they mix with the other components and can be converted into coatings by conventional coating methods. Some examples of homopolymers and copolymers are those of olefins, acrylates, methacrylates, vinyl esters, acrylonitrile, dienes, styrene, methylstyrene, vinyl chloride, vinyl fluoride, vinylidene chloride and vinyl ethers. Some specific examples are polyvinyl chloride, polyvinylidene chloride, polyvinyl fluoride, polyacrylonitrile, polystyrene, poly(methylstyrene), polyacrylates and polymethacrylates containing C₁-C₁₈alkyl radicals in the ester groups, vinyl chloride-vinyl acetate copolymers, vinyl chloride-vinyl acetate-vinyl alcohol copolymers, vinyl chloride-vinyl acetate-acrylonitrile copolymers, vinyl chloride-vinylidene chloride copolymers, vinylidene chloride-acrylonitrile copolymers, and acrylonitrile-butadiene-styrene copolymers. Preferred polymers are homopolymers of vinyl chloride and vinylidene chloride and copolymers of vinyl chloride and/or vinylidene chloride and acrylates, methacrylates, vinyl esters, vinyl alcohol, acrylonitrile and styrene. Particular preference is given to polyvinyl chloride.

It is advantageous to incorporate a plasticizer into the polymers in order to optimize the diffusion coefficients of the membrane components for ion exchange. Depending on the type of polymer and plasticizer, from 10 to 90 % by weight, preferably from 20 to 70 % by weight, particularly preferably from 20 to 50 % by weight, of polymer and from 90 to 10 % by weight, preferably from 80 to 30 % by weight, in particular from 80 to 50 % by weight, of plasticizer may be present. Suitable plasticizers are known in large number. They can be, for example, higher alkanols or esters thereof, esters of fatty acids with diols or alkanols, ethers with higher alkanols, esters of di- and polycarboxylic acids and esters of higher alkanols and phosphoric acid or phosphorous acid. The higher alkanols can be, for example, linear or branched C₆-C₂₂alkanols or phenols substituted by 1 to 3 linear or branched C₃-C₁₈alkyl or C₃-C₁₈alkoxy groups. Some specific examples are octadecanol, phthalic diesters, glutaric diesters, adipic diesters, azelaic diesters and sebacic diesters with C₈-C₁₈alkanols, such as 2-ethylhexanol, n-octanol, n-decanol, n-dodecanol, tetradecanols, hexadecanols, heptadecanois and octadecanols, tris(2-ethylhexyl) phosphate or tris(2-ethylhexyl) phosphite, tris(nonylphenyl) phosphite, ethylene glycol monostearate, ethylene glycol dibenzoate and 2-nitrophenylbutyl or -octyl ether. The type and amount of plasticizers are expediently selected so that they are compatible with the polymer, so that phase separations do not occur and so that the hydrophobic properties of the polymer undergo little or no change. The plasticizers should be lipophilic and contribute to dissolution and distribution of the components in the coating (matrix).

Examples of suitable salts with lipophilic anions are alkali metal, alkaline earth metal and ammonium salts with substituted or unsubstituted tetraphenylborates. Preferred cations are Li^{\oplus} , Na^{\oplus} , K^{\oplus} , $\text{Mg}^{2\oplus}$, $\text{Ca}^{2\oplus}$, NH_4^{\oplus} , and the ammonium cations of primary, secondary and tertiary amines and quaternary ammonium cations which can contain from 1 to 60 carbon atoms. Some examples of ammonium cations are methyl-, ethyl-, propyl-, butyl-, hexyl-, octyl-, decyl-, dodecyl-, tetradecyl-, hexadecyl-, octadecyl-, dimethyl-, diethyl-, dibutyl-, butylmethyl-, dioctyl-, didodecyl-, dodecylmethyl-, trimethyl-, triethyl-, tripropyl-, tributyl-, trioctyl-, tridodecyl-, dodecyldimethyl-, didodecylmethyl-, tetramethyl-, tetraethyl-, tetrapropyl-, tetrabutyl-, tetrahexyl-, tetraoctyl-, tetradecyl-, tetradodecyl-, dodecyltrimethyl-, octyltrimethyl-, didodecyltrimethyl-, tridodecyltrimethyl-, tetradecyltrimethyl- and octadecyltrimethylammonium. Quaternary ammonium salts are preferred, in particular those having 4 to 48 carbon atoms.

An example of a suitable borate anion is tetraphenylborate, whose phenyl groups may be substituted by one or more, preferably 1 to 3, particularly preferably 1 or 2, $\text{C}_1\text{-C}_4$ alkyl, $\text{C}_1\text{-C}_4$ alkoxy, halogen, for example F or Cl, or trifluoromethyl groups. Some specific examples are sodium tetraphenylborate, sodium tetra(3,5-bistrifluoromethylphenyl)borate, potassium tetra(4-chlorophenyl)borate, tetrabutylammonium tetraphenylborate and tetradodecyl(4-chlorophenyl)borate. The salts with lipophilic anions serve as negative charge compensation for the metal cations diffusing into the active coating and to be measured therein in complexed form.

The amount of salts with lipophilic anions can be, for example, from 0.01 to 10 % by weight, preferably from 0.1 to 5 % by weight, particularly preferably from 0.1 to 2 % by weight, based on the amount of polymer and plasticizer.

The polymer coating (also referred to as membrane) contains an ionophore in, for example, an amount of from 0.01 to 10 % by weight, preferably from 0.1 to 5 % by weight, particularly preferably from 0.1 to 2 % by weight, based on the amount of polymer and plasticizer. Ionophores are natural or synthetic organic compounds which contain a plurality of, usually alternating, electron-rich heteroatoms, for example S, N and in particular O, in linear or cyclic carbon chains and which are capable of selectively complexing the metal cations to be measured. The natural compounds are frequently macrocyclic compounds, for example valinomycin, which is capable of selectively binding potassium cations. Another example is nonactin. A large group of ionophores comprises macrocyclic polyethers (crown ethers), which are capable of complexing

various metal cations, depending on the geometry and size. Further examples of ionophores are coronandenes, kryptandenes and calixarenes. Examples of linear ionophores are podandenes. Such ionophores are described, for example, in US-A-4 645 744.

The amount of compounds of the formulae I and II can be, for example, from 0.01 to 10 % by weight, preferably from 0.1 to 5 % by weight, particularly preferably from 0.1 to 2 % by weight, based on the amount of polymer and plasticizer. The compounds of the formulae I and II can also be bonded to the polymers via suitable bridging groups.

The fluorophores to be used according to the invention have very suitable absorption and emission wavelength ranges which allow the use of known and inexpensive light sources and detectors, for example halogen or xenon lamps or light-emitting diodes. Examples of detectors which can be employed are photodiodes. The fluorophores furthermore have high absorption coefficients and high quantum yields can be achieved. The high lipophilicity, high basicity and the large dynamic range of the change in fluorescence between the protonated and deprotonated forms satisfy, in particular, the high requirements for optical determination of cations based on fluorescence measurements.

Examples of suitable cations are metal cations of metals from the first to fifth main groups and the first to eighth sub-groups of the Periodic Table of the Elements, the lanthanides and actinides. Some examples of metals are Li, Na, K, Rb, Cs, Mg, Ca, Sr, Ba, B, Al, Ga, In, Tl, Sn, Pb, Sb, Bi, Cu, Ag, Au, Zn, Cd, Hg, Sc, Y, Ti, Zr, Hf, Cr, Mo, W, Mn, Fe, Co, Ni, Ru, Os, Rh, Ir, Pt, Pd, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Yb, Lu, Ac, Th, Pa, U, Np and Pu. Preferred metal cations are the alkali and alkaline earth metal ions, in particular Li^{\oplus} , Na^{\oplus} , K^{\oplus} , $\text{Mg}^{2\oplus}$, $\text{Ca}^{2\oplus}$ and $\text{Sr}^{2\oplus}$, very particularly K^{\oplus} , Na^{\oplus} and Ca^{\oplus} . Examples of ammonium cations are NH_4^{\oplus} and the cations of protonated primary, secondary and tertiary amines and quaternary ammonium. The amines can contain from 1 to 40, preferably from 1 to 20, particularly preferably from 1 to 12, carbon atoms. The quaternary ammonium can contain from 4 to 40, preferably from 4 to 20, particularly preferably from 4 to 16, carbon atoms.

The composition according to the invention is highly suitable as an optical sensor for the quantitative determination of cations, in particular metal cations, very particularly potassium cations, in an aqueous environment, preferably by means of fluorescence spectrometry. The determinations can be carried out quickly with high accuracy even for

low concentrations (for example in the micromolar range to the nanomolar range), since the pH-dependent equilibria of the complexing reactions and of proton exchange become established quickly and the fluorophores are characterized by a high fluorescence quantum yield and sensitivity. The analyses can be carried out, for example, directly in body fluids (blood, urine, serum), natural water or waste water, where it may be possible for any interfering cations to be specifically bound or removed in advance. The composition according to the invention is particularly suitable for the determination of physiological amounts, for example in the range from 0.5 to 10 mmol, of cations in aqueous media.

In addition to the preferred method of fluorescence spectroscopy, other optical measurement methods may also be used, for example surface plasmon resonance spectroscopy, absorption spectroscopy, reflection spectroscopy, interferometry or surface-amplified Raman or fluorescence spectroscopy.

The invention furthermore relates to an optical sensor for the determination of cations in aqueous measurement samples, in particular by means of fluorescence spectrometry, which comprises

- (a) a transparent support,
- (b) which is coated on at least one side with a transparent coating comprising
 - (b1) a hydrophobic polymer,
 - (b2) a plasticizer,
 - (b3) the salt of a lipophilic anion,
 - (b4) an ionophore which forms a complex with the ion to be determined, and
 - (b5) a compound of the formula I or II as the fluorophore.

The invention furthermore relates to a method for the optical determination of cations in aqueous measurement samples, in which a composition according to the invention is brought into contact with said aqueous measurement sample, and then, in particular, the reduction in fluorescence in the active polymer coating is measured.

The invention furthermore relates to the use of the compounds of the formulae I and II as fluorophores for the optical determination of cations in aqueous measurement samples.

The process according to the invention can be carried out, for example, by immobilizing the composition according to the invention comprising support and active polymer coating in an optical cell in which the active coating is brought into contact with the measurement

sample. The optical cell furthermore contains a window through which the active coating can be excited by irradiation and the emitted fluorescence radiation can be measured by means of a spectrophotometer. The wavelengths are adjusted so that the absorption is at a maximum for the irradiation and the emission is at a maximum for the fluorescence measurement. The decrease in intensity as a function of time is measured. The measurement system can be designed so that the measurement is carried out discontinuously or continuously, for example by pumping the measurement solution through the measurement cell. In order to determine unknown concentrations of cations, the system is first calibrated by means of measurement samples of known concentration, and the concentrations are plotted as a function of the fluorescence intensity. It is expedient to add pH buffers to the measurement sample, since the sensitivity of the measurement, and consequently also the fluorescence intensity of the fluorophore, depends on the pH of the measurement solution due to the pH-dependence of the absorption spectrum. The pH range of the measurement sample can be, for example, from 4 to 8, more preferably from 5.5 to 6.5. Examples of suitable buffers are citrate buffers and phosphate buffers. Further buffer systems are described in US-A-4 645 744, in particular including those which are incorporated directly into the active coating in order to avoid addition to the measurement sample.

The examples below illustrate the invention in greater detail.

A) Preparation of fluorophores

Example A1: Preparation of 3,6-bis(n-octylamino)acridine.

6.33 g of anhydrous potassium carbonate are added to a solution of 2.5 g of 3,6-diaminoacridine hydrochloride and 3.55 ml of 1-bromooctane in 50 ml of dimethyl sulfoxide, and the mixture is stirred at 80°C for 48 hours. The cooled reaction mixture is subsequently poured onto ice, and the brown suspension is extracted with methylene chloride. The organic phase is washed with saturated aqueous NaCl solution and dried over sodium sulfate. After evaporation, the red-brown oil is chromatographed on silica gel using methylene chloride/methanol (9:1). After evaporation of the solvent, the residue is taken up in diethyl ether/methanol (10:1) and chromatographed on aluminium oxide. The eluate is taken up in methanol, 2N HCl is added, the mixture is extracted with diethyl ether, and the ether phase is dried and evaporated. The residue is dissolved in methylene chloride, n-hexane is added, and the red crystalline precipitate formed is filtered off. Further product can be isolated from the mother liquor after evaporation. The melting

point of the title compound is 245°C. Absorption spectrum (ethanol): $\lambda_{\text{max}} = 472 \text{ nm}$; $\epsilon = 51\,400$.

Example A2: Preparation of 3,6-bis(n-eicosylamino)acridine.

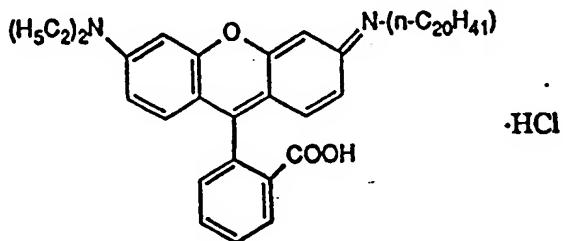
2.53 g of anhydrous potassium carbonate are added to a solution of 2.5 g of 3,6-diaminoacridine hydrochloride and 2.95 g of 1-eicosyl bromide in 20 ml of N,N'-dimethylethyleneurea, and the mixture is stirred at 50°C for 86 hours. The cooled reaction mixture is subsequently poured into water, and the orange-brown suspension is extracted with methylene chloride. The organic phase is washed with water and dried over sodium sulfate. After evaporation, 2N HCl is added to the brown oil. The red precipitate formed is filtered off, washed with water and then dried in a high vacuum. The resultant red-brown crystals are taken up in methylene chloride/methanol (10:1) and chromatographed on silica gel. After evaporation, the residue is taken up in diethyl ether/methanol (10:1) and re-chromatographed on silica gel, giving the title compound as red crystals, absorption spectrum (ethanol): $\lambda_{\text{max}} = 472 \text{ nm}$; $\epsilon = 42\,200$.

Example A3: Preparation of 3,6-bis(n-hexylamino)acridine.

298 mg of ground potassium hydroxide are added to a solution of 500 mg of N,N'-bisostyl-3,6-diaminoacridine and 797 mg of 1-bromohexane in 25 ml of dimethylformamide, and the mixture is stirred at 60°C for 22 hours. The cooled reaction mixture is subsequently poured into water and extracted with ethyl acetate, and the organic phase is separated off, washed with aqueous NaCl solution and dried over sodium sulfate. Evaporation gives a dark-red oil, which is taken up in toluene/ethyl acetate (20:1) and chromatographed on silica gel. Evaporation of the solvent gives a yellow, viscous oil, which is dissolved in 11.5 ml of glacial acetic acid, 4.6 ml of 97 % sulfuric acid are added with water cooling, and the mixture is then stirred at room temperature for 15 hours. The red reaction mixture is poured into ice water and adjusted to pH 11 by means of 30 % NaOH. The mixture is extracted with ethyl acetate, and the organic phase is washed with 2N HCl and saturated aqueous NaCl solution and then dried over sodium sulfate. After evaporation, the dark-red, viscous oil is taken up in t-butyl methyl ether/methanol (5:1) and chromatographed on silica gel, giving the title compound as orange-red crystals having a melting point of > 200°C (decomposition). $^1\text{H-NMR}$ (CDCl_3): 8.1 [s, 1H, C(9)]; 7.44 [d, 2H, C(8)]; 6.93 [s, 2H, C(5)]; 6.82 [d, 2H, C(7)]; 3.20 [t, 4H, N- CH_2]; 1.68 [m, 6H, CH_3].

Example A4: Preparation of 3,6-bis(n-heptylcarbonylamino)acridine.

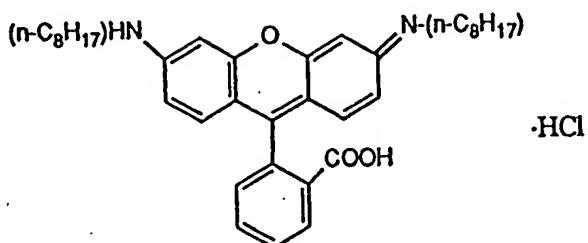
3.1 ml of heptanoyl chloride are slowly added dropwise to a suspension of 2.5 g of 3,6-diaminoacridine hydrochloride in 50 ml of pyridine, and the mixture is then stirred for 30 minutes. The reaction mixture is subsequently poured into water. The yellow suspension is extracted with methylene chloride, and the organic phase is washed with aqueous saturated NaCl solution and dried over sodium sulfate. After evaporation, the dark-red oil is taken up in methylene chloride/methanol (10:1) and chromatographed on silica gel. The evaporated eluate is taken up in methylene chloride and added dropwise to cyclohexane. The yellow precipitate formed is filtered off, washed with cyclohexane and dried in a high vacuum, giving the title compound as yellow crystals having a melting point of 243-244°C. Absorption spectrum (ethanol): $\lambda_{\text{max}} = 384 \text{ nm}$; $\epsilon = 2300$.

Example A5: Preparation of

- a) A solution of 12.8 g of phthalic anhydride and 13.2 g of 3-N,N-diethylaminophenol is stirred at 110°C for 16 hours in 75 ml of toluene. The precipitated product is filtered off and recrystallized from ethanol, giving brick-red crystals of 1-carboxy-1'-hydroxy-3'-diethylaminobenzophenone (product A) having a melting point of 214°C.
- b) A solution of 5.5 g of 3-aminophenol and 21.6 g of 1-bromoeicosane in 250 ml of 1,4-dioxane is stirred at 100°C for 48 hours. The mixture is evaporated in vacuo, and the brown, gelatinous residue is taken up in toluene/ethyl acetate (10:1) and chromatographed on silica gel, giving 3-N-eicosylaminophenol as white crystals having a melting point of 80°C.
- c) 626 mg of product A and 790 mg of 3-N-eicosylaminophenol are stirred for 2 hours at 170°C in 5 ml of phosphoric acid (85%). After cooling, a solution of 1 ml of concentrated HCl in 1 ml of methanol is added, and the mixture is subsequently extracted with methylene chloride. After removal of the solvent, the residue is taken up in methylene chloride/methanol (85:15) and chromatographed on silica gel, giving the title compound as

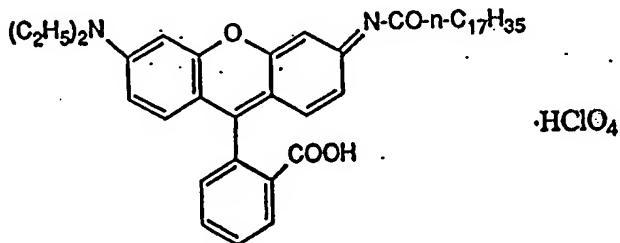
red-violet crystals having a melting point of 115°C. Absorption spectrum (ethanol):
 $\lambda_{\text{max}} = 532 \text{ nm}; \epsilon = 90\,000.$

Example A6: Preparation of



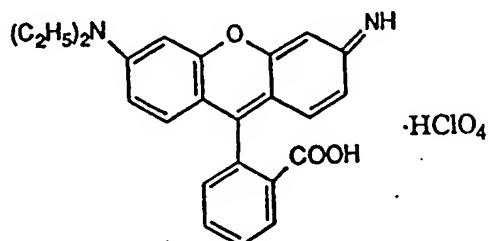
- a) A solution of 5.45 g of 3-aminophenol and 11.6 g of 1-bromoocetane in 250 ml of dioxane is stirred at 100°C for 80 hours, the solvent is then evaporated, and the residue is then taken up in toluene/ethyl acetate (10:1) and chromatographed on silica gel, giving N-octylaminophenol as beige crystals, melting point 75°C.
 b) 1.1 g of N-octylaminophenyl and 0.37 g of phthalic anhydride are melted together at 100°C. 1 ml of phosphoric acid (85 %) is added to the melt, which is then heated to 170°C. After 1 hour, the mixture is allowed to cool, and 2N HCl is added. The mixture is extracted with methylene chloride, the solvent is removed, and the red residue is taken up in methylene chloride/methanol (85:15). Chromatography on silica gel gives the title compound as red crystals having a melting point of 183°C. Absorption spectrum (ethanol):
 $\lambda_{\text{max}} = 522 \text{ nm}; \epsilon = 73\,700.$

Example A7: Preparation of



- a) 1.57 g of product A from Example A5a, 0.55 g of 3-aminophenol and 10 ml of phosphoric acid (85 %) are stirred for 30 minutes at 170°C. 6.7 ml of perchloric acid (50 %) and 100 ml of methanol are then added, the mixture is re-heated, and the solvent is

then removed in vacuo. The residue is taken up in methylene chloride, the solution is washed with water, and the solvent is removed again. The residue is taken up in methylene chloride/methanol (10:1) and chromatographed on silica gel, giving red crystals of compound B of the formula



having a melting point of 175°C.

b) 0.1 g of compound B is dissolved in 1 ml of methylene chloride and 0.3 ml of pyridine, and 100 mg of stearoyl chloride are added. After 3 hours, the mixture is evaporated to dryness in vacuo, and the residue is dissolved in methylene chloride/methanol (85:15) and chromatographed on silica gel, giving the title compound as red crystals having a melting point of 145°C. Absorption spectrum (ethanol): $\lambda_{\text{max}} = 56 \text{ nm}$; $\epsilon = 10\,900$.

B) Production of coated supports

Examples B1-B6:

a) The support material used is pretreated glass. Circular glass sheets (diameter 18 mm, thickness 0.17 mm) are immersed for one hour in a solution of 10 % by volume of dimethyldodecylchlorosilane in isopropanol. The glass sheets are then each washed one after the other with 200 ml of isopropanol, ethanol and methanol and dried at 110°C for 1 hour. The hydrophobicized surface has better adhesion of the membrane coating.

b) Preparation of the coating solution.

The following constituents are introduced into a 2 ml bottle together with 1.5 ml of tetrahydrofuran and shaken until the components have dissolved:

1. 80 mg of polyvinyl chloride (Fluka, 81392)
2. 160 mg of bis(2-ethylhexyl) sebacate (plasticizer)
3. 5 mg of valinomycin
4. 1.5 mg of potassium tetrakis(4-fluorophenyl)borate
5. 2 mg of fluorophore

<u>Example No.</u>	<u>Fluorophore</u>
B1	Example A5
B2	Example A6
B3	Example A7
B4	Example A1
B5	Example A2
B6	Example A4

c) Production of coated glass supports.

The glass supports are clamped in the chamber of a spin-coating apparatus (Optocoat OS 35var, Willer Company, CH-8484 Weisslingen). The chamber is rinsed with 10 ml of tetrahydrofuran and rotated for 2 minutes at 3 800 revolutions/minute. 50 µl of the particular coating solution are then pipetted onto the glass support, and the glass support is rotated for a further 10 seconds. The glass support coated with a membrane is then removed and dried for 10 minutes in air.

d) Spectral properties of the membranes.

The coated glass supports are clamped in an optical cell in which the membrane is in contact with the measurement liquid. The membrane can be optically excited in the optical cell and the fluorescence radiation measured. The optical cell is introduced into a spectrophotometer (Perkin-Elmer LS-50), and the absorption and emission spectra are measured. The relative fluorescence quantum yield is furthermore determined by the method described by Calvert and Pitts in Photochemistry, pages 799 to 805 (1966); John Wiley and Sons Inc., using fluorescein as standard. The results are shown in Table 1.

Table 1:

<u>Example</u>	Absorption (λ_{max} , nm)	Emission (λ_{max} , nm)	Absorbance coefficient	Quantum yield
B1	540	570	90 000	0.14
B2	525	555	73 700	0.33
B3	500	560	20 000	0.08
B4	470	505	51 400	0.75
B5	470	505	42 200	0.75
B6	380	460	2 300	0.10

Examples B7 to B12: Determination of pK_a values and the change in fluorescence.

The fluorophores are dissolved in a mixture of 30 % by volume of methanol and 70 % by volume of phosphate buffer and adjusted to various pH values between 6 and 13. The measurement solutions are introduced into a cell, and the fluorescence intensity is measured using a spectrophotometer. The fluorescence intensity is plotted as a function of pH, and the pK_a value is determined from the point of inflection in the curve. The ratio between the protonated and deprotonated forms of the fluorophore is furthermore determined from the change in fluorescence by dissolving the fluorophore in tetrahydrofuran, adjusting the pH by addition of 1M HCl or 1.0M NaOH and determining the change in fluorescence intensity in percent. The concentration of the fluorophores is 10⁻⁷ mol/l in each case. The results are shown in Table 2.

Table 2:

<u>Fluorophore of Example</u>	<u>pK_a value</u>	<u>Change in fluorescence intensity</u>
A1	10.0	44
A2	11.1	41
A4	10.0	24
A5	10.3	33
A6	12.0	20
A7	10.1	15

C) Use examples:

Example C1: The same experimental set-up as in Example B1d is used and the absorption and emission wavelengths are adjusted to the corresponding maxima of the fluorophores employed in the membrane. The membrane is brought into contact with an aqueous KCl solution of defined concentration by pumping the solution through the cell at a rate of 1 ml/min and determining the change in fluorescence intensity. Before the measurement and after each measurement, the cell is rinsed with potassium ion-free buffer solutions and the fluorescence intensity is determined in order to define the base line. The reduction in fluorescence intensity in percent at the respective potassium concentrations for the fluorophore of Example A5 (membrane B1) is shown in Table 3.

Table 3:

<u>Potassium concentration (mM)</u>	<u>% reduction in fluorescence intensity</u>
0.01	5
0.1	20
0.5	35
1.0	45
5.0	53
10.0	60
100.0	68

It can be seen from the table that the fluorescence intensity decreases with increasing potassium concentration and, after calibration, unknown concentrations can be determined with high reliability. In particular in the physiological range from about 0.5 to 10 mM of potassium, high sensitivity is guaranteed.

Example C2: The procedure is as in Example C1 using the fluorophore of Example A6. The reduction in fluorescence intensity in percent and the respective potassium concentrations for the fluorophore of Example A6 (membrane B2) are shown in Table 4.

Table 4:

<u>Potassium concentration (mM)</u>	<u>% reduction in fluorescence intensity</u>
0.1	36
0.5	41
1.0	43
5.0	48
10.0	52

Example C3: The procedure is as in Example C1 using the fluorophore of Example A1. The reduction in fluorescence intensity in percent and the respective potassium concentrations for the fluorophore of Example A1 (membrane B4) are shown in Table 5.

Table 5:

<u>Potassium concentration (mM)</u>	<u>% reduction in fluorescence intensity</u>
0.1	5
0.5	15
1.0	25
5.0	39
10.0	45
100.0	75

Example C4: The procedure is as in Example C1 and the sodium ion concentration is determined using a membrane comprising 2 mg of fluorophore of Example A5, 30 mg of sodium ionophore (4-octadecanoyloxymethyl-N,N,N,N-tetracyclohexyl-1,2-phenylenedioxydiacetamide, Fluka sodium ionophore V, Catalogue No. 71738), 2 mg of potassium tetrakis(4-chlorophenyl)borate, 75 mg of polyurethane (Tecoflex®), 160 mg of bis-2-ethylhexyl sebacate as plasticizer and 1 ml of tetrahydrofuran. The sensor is exposed to a buffer solution at pH 5 (0.1 mol of trishydroxymethylaminomethane and 1M HCl) with different sodium concentrations. The results are shown in Table 6.

Table 6:

<u>Sodium concentration (mM)</u>	<u>% reduction in fluorescence intensity</u>
0	0
3	15
15	41
30	54
90	72
150	79
210	83
300	86

Example C5: The procedure is as in Example C1 and the calcium ion concentration is determined using a membrane comprising 2 mg of fluorophore of Example A5, 30 mg of calcium ionophore ((--)(R,R)-N,N-[bis(11-ethoxycarbonyl)undecyl]-N,N-4,5-tetramethyl-3,6-dioxaoctanediamide, Fluka calcium ionophore I, Catalogue No. 21192), 6 mg of potassium tetrakis(4-chlorophenyl)borate, 75 mg of polyurethane (Tecoflex®),

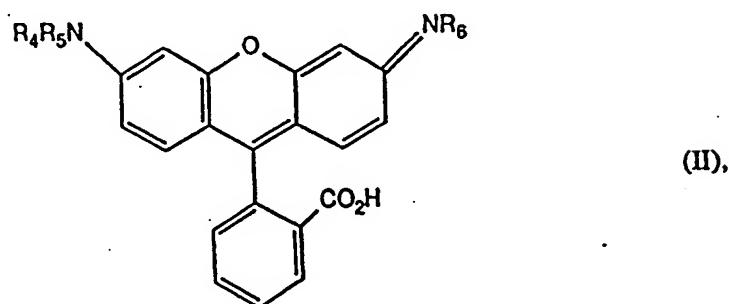
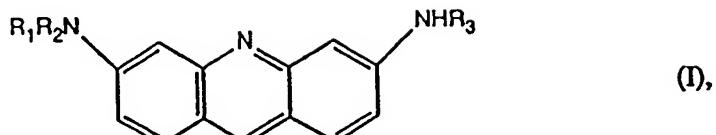
160 mg of bis-2-ethylhexyl sebacate as plasticizer and 1 ml of tetrahydrofuran. The sensor is exposed to a buffer solution at pH 5 (0.02M NaOH and acetic acid) with different calcium concentrations. The results are shown in Table 7.

Table 7:

<u>Calcium concentration (mM)</u>	<u>% reduction in fluorescence intensity</u>
0	0
0.1	26.5
0.5	43.1
1.0	50.6
3.0	61.0
5.0	65.8
7.0	68.1
10.0	71.0

WHAT IS CLAIMED IS:

1. A compound of the formula I or II



in which R₁ and R₃, and R₄ and R₆ are C₁-C₃₀alkyl or C₁-C₃₀alkyl-CO-, and R₂ and R₅ are H or C₁-C₃₀alkyl, with the proviso that the total number of carbon atoms in the alkyl groups is at least 12, or a salt thereof with inorganic or organic acid.

2. A compound according to claim 1, wherein R₂ is H.

3. A compound according to claim 1, wherein the alkyl groups are linear alkyl groups.

4. A compound according to claim 1, wherein the alkyl groups contain 1 to 22 carbon atoms.

5. A compound according to claim 1, wherein R₁ and R₃ are C₆-C₂₄alkyl or C₆-C₂₄alkyl-CO-, and R₂ is H.

6. A compound according to claim 5, wherein R₁ and R₃ are C₁₀-C₂₄alkyl or C₁₀-C₂₄alkyl-CO-.

7. A compound according to claim 5, wherein R₁ and R₃ are C₁₄-C₂₂alkyl or C₁₄-C₂₂alkyl-CO-.

8. A compound according to claim 1, wherein R₅ is H and R₄ and R₆ are C₆-C₂₄alkyl.

9. A compound according to claim 8, wherein R₄ and R₆ are C₁₀-C₂₄alkyl.

10. A compound according to claim 9, wherein R₄ and R₆ are C₁₄-C₂₂alkyl.

11. A compound according to claim 1, wherein R₄ and R₅ are C₁-C₆alkyl, and R₆ is C₁₀-C₂₄alkyl or C₁₀-C₂₄alkyl-CO-.

12. A compound according to claim 11, wherein R₄ and R₅ are C₁-C₄alkyl, and R₆ is C₁₄-C₂₂alkyl or C₁₄-C₂₂alkyl-CO-.

13. A compound according to claim 12, wherein R₄ and R₅ are methyl or ethyl, and R₆ is C₁₆-C₂₂alkyl or C₁₆-C₂₂alkyl-CO-.

14. A compound according to claim 1, wherein the salt of the compound of the formula I or II is derived from HF, HCl, HBr, HI, H₂SO₃, H₂SO₄, H₃PO₃, H₃PO₄, HNO₂, HNO₃, HClO₄, HBF₄, HPF₆, HSbF₆, CF₃SO₃H, toluenesulfonic acid, C₁-C₄alkyl- or phenylphosphonic acid, formic acid, acetic acid, propionic acid, benzoic acid, mono-, di- or trichloroacetic acid, or mono-, di- or trifluoroacetic acid.

15. A compound according to claim 14, wherein the salt of the compound of the formula I or II is derived from HCl, HBr, H₂SO₄, HClO₄, HBF₄, HPF₆ or HSbF₆.

16. A composition comprising

- (a) a transparent support,
- (b) which is coated on at least one side with a transparent coating which comprises,
 - (b1) a hydrophobic polymer,
 - (b2) a plasticizer,
 - (b3) the salt of a lipophilic anion,
 - (b4) an ionophore which forms a complex with the ion to be determined, and
 - (b5) a compound of the formula I or II as fluorophore.

17. A composition according to claim 16, wherein the compound of the formula I or II has a pK_a value of at least 8.

18. A composition according to claim 17, wherein the pK_a value is at least 10.
19. A composition according to claim 16, wherein the support is a glass.
20. A composition according to claim 16, wherein the thickness of the coating on the support can be, for example, from 0.01 to 100 µm.
21. A composition according to claim 16, wherein the hydrophobic polymer has a molecular weight of at least 100 000 daltons.
22. A composition according to claim 16, wherein the hydrophobic polymer is a homopolymer or copolymer of olefins, acrylates, methacrylates, vinyl esters, acrylonitrile, dienes, styrene, methylstyrene, vinyl chloride, vinyl fluoride, vinylidene chloride and/or vinyl ether.
23. A composition according to claim 22, wherein the hydrophobic polymer is polyvinyl chloride, polyvinylidene chloride, polyvinyl fluoride, polyacrylonitrile, polystyrene, poly(methylstyrene), a polyacrylate or a polymethacrylate containing C₁-C₁₈alkyl radicals in the ester group, a vinyl chloride-vinyl acetate copolymer, a vinyl chloride-vinyl acetate-vinyl alcohol copolymer, a vinyl chloride-vinyl acetate-acrylonitrile copolymer, a vinyl chloride-vinylidene chloride copolymer, a vinylidene chloride-acrylonitrile copolymer or an acrylonitrile-butadiene-styrene copolymer.
24. A composition according to claim 22, wherein the hydrophobic polymer is a homopolymer made from vinyl chloride and vinylidene chloride or a copolymer made from vinyl chloride and/or vinylidene chloride and an acrylate, methacrylate, vinyl ester, vinyl alcohol, acrylonitrile or styrene.
25. A composition according to claim 24, wherein the hydrophobic polymer is polyvinyl chloride.
26. A composition according to claim 16, wherein the plasticizer is present in an amount of from 10 to 90 % by weight, based on the polymer.
27. A composition according to claim 16, wherein the plasticizer is a higher alkanol or an

ester thereof, an ester of a fatty acid with a diol or alkanol, an ether with a higher alkanol, an ester of a di- or polycarboxylic acid or an ester of a higher alkanol and phosphoric acid or phosphorous acid.

28. A composition according to claim 16, wherein the salt with a lipophilic anion is an alkali metal, alkaline earth metal or ammonium salt with a substituted or unsubstituted tetraphenylborate.

29. A composition according to claim 28, wherein the cation is Li^{\oplus} , Na^{\oplus} , K^{\oplus} , $\text{Mg}^{2\oplus}$, $\text{Ca}^{2\oplus}$, NH_4^{\oplus} or an ammonium cation of a primary, secondary or tertiary amine or a quaternary ammonium cation containing 1 to 60 carbon atoms.

30. A composition according to claim 28, wherein the borate anion is tetraphenylborate, whose phenyl groups are unsubstituted or substituted by one or more $\text{C}_1\text{-C}_4$ alkyl, $\text{C}_1\text{-C}_4$ alkoxy, halogen or trifluoromethyl groups.

31. A composition according to claim 28, wherein the borate anion is sodium tetraphenylborate, sodium tetra(3,5-bistrifluoromethylphenyl)borate, potassium tetra(4-chlorophenyl)borate, tetrabutylammonium tetraphenylborate or tetradodecyl(4-chlorophenyl)borate.

32. A composition according to claim 16, wherein the amount of the salt with a lipophilic anion is from 0.01 to 10 % by weight, based on the amount of polymer and plasticizer.

33. A composition according to claim 16, wherein the polymer coating contains an ionophore in an amount of from 0.01 to 10 % by weight, based on the amount of polymer and plasticizer.

34. A composition according to claim 16, wherein the ionophore is valinomycin, which is capable of selectively binding potassium cations.

35. A composition according to claim 16, wherein the amount of the compound of the formula I or II is from 0.01 to 10 % by weight, based on the amount of polymer and plasticizer.

36. A composition according to claim 35, wherein the amount of the compound of the

formula I or II is from 0.1 to 5 % by weight.

37. A composition according to claim 35, wherein the amount of the compound of the formula I or II is from 0.1 to 2 % by weight.

38. An optical sensor for the determination of cations in aqueous measurement samples, in particular by means of fluorescence spectrometry, which comprises

- (a) a transparent support,
- (b) which is coated on at least one side with a transparent coating which comprises
 - (b1) a hydrophobic polymer,
 - (b2) a plasticizer,
 - (b3) the salt of a lipophilic anion,
 - (b4) an ionophore which forms a complex with the ion to be determined, and
 - (b5) a compound of the formula I or II as fluorophore.

39. A method for the optical determination of cations in aqueous measurement samples, in which a composition according to claim 16 is brought into contact with said aqueous measurement sample, and the reduction in fluorescence in the active polymer coating is then measured.

40. The use of a compound of the formula I or II as a fluorophore for the optical determination of cations in aqueous measurement samples.

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